

Characterization of non -Shiga toxin- producing *Escherichia coli* O157 strains isolated from dogs

A. BENTANCOR^{1*}, D. A. VILTE², M. V. RUMI¹, C. C. CARBONARI³, I. CHINEN³; M. LARZÁBAL⁴,
A. CATALDI⁴, E. C. MERCADO²

¹Microbiología, Facultad de Ciencias Veterinarias, Universidad de Buenos Aires. Chorroarín 280 (1427) Ciudad Autónoma de Buenos Aires; ²Instituto de Patobiología and ⁴Instituto de Biotecnología, Instituto Nacional de Tecnología Agropecuaria (INTA). Los Reseros y Las Cabañas (1712) Castelar, Provincia de Buenos Aires; ³Servicio de Fisiopatología, INEI-ANLIS "Dr. Carlos G. Malbrán". Av. Vélez Sarsfield 563 (1281) Ciudad Autónoma de Buenos Aires, Argentina.

*Correspondence. E-mail: aben@fvet.uba.ar

ABSTRACT

Shiga toxin-negative *Escherichia coli* O157 strains of various H types have been associated with diarrhea in children and are considered potentially pathogenic for humans. In this study, we describe non-Shiga toxin-producing *E. coli* O157 *E. coli* strains previously obtained from dogs in Argentina. Different *E. coli* phylogenetic lineages corresponding to flagellar types H16, H29 and H45 were identified. *E. coli* serotypes O157:H16 and O157:H45 contained intimin subtypes ϵ and α_1 , respectively. Serotype O157:H45 carried the *bfp* gene encoding the bundle-forming pilus. Localized adherence-like patterns to HEp-2 cells were observed in O157:H16 strains, while O157:H45 adhered in a typical localized pattern. A total of eight different *Xba*I-pulse field electrophoresis patterns with more than 74 % similarity were identified among the nine *E. coli* O157:H16 strains. Our data emphasized the fact that dogs may harbor human pathogenic *E. coli* O157 which do not correspond to Shiga toxin-producing strains and whose potential human health hazard should not be underestimated.

Key words: *Escherichia coli*, O157, non-Stx, dogs

RESUMEN

Caracterización de cepas de *Escherichia coli* O157 no productoras de toxina Shiga aisladas de perros. Cepas de *E. coli* O157 no productoras de toxina Shiga (Stx) que presentan diversos antígenos flagelares han sido aisladas de niños con diarrea y se consideran potencialmente patógenas para humanos. En el presente trabajo se describen cepas Stx-negativas de *E. coli* O157 de distintos tipos flagelares previamente aisladas de perros en Argentina. Los tipos flagelares H16, H29 y H45 correspondieron a diferentes grupos filogenéticos. Los serotipos O157:H16 y O157:H45 presentaron los subtipos de intimina ϵ y α_1 , respectivamente. En el serotipo O157:H45 se detectó la presencia del gen *bfp*, codificante de la fimbria *bundle-forming pili*. El patrón de adherencia en células HEp-2 correspondió al tipo similar a localizada para *E. coli* O157:H16, mientras que O157:H45 mostró adherencia localizada típica. Dentro de las 9 cepas de *E. coli* O157:H16 estudiadas se detectaron 8 patrones de *Xba*I-PFGE con más del 74% de similitud. Nuestros datos confirman que los perros pueden ser portadores de *E. coli* O157 patógenas no productoras de Stx, las que representan un riesgo para la salud pública que no debe ser subestimado.

Palabras clave: *Escherichia coli*, O157, No-Stx, perros

Attaching and effacing *Escherichia coli* (AEEC) are a group of *Escherichia coli* strains that are able to colonise the intestinal mucosa of humans and animals with a characteristic histopathological lesion known as attaching and effacing (A/E). A/E lesions are initiated by the attachment of bacteria to the enterocytes, a process mediated by intimin, an outer membrane adhesin encoded by the *eae* gene. Intimin and most of the proteins responsible for A/E lesions are encoded by genes clustered in a pathogenicity island, referred to as the locus of enterocyte effacement (LEE) (9).

Some AEEC strains also produce Shiga toxins and these strains are called enterohemorrhagic *E. coli* (EHEC).

On the other hand, enteropathogenic *E. coli* (EPEC) is defined as AEEC strains which possess the EPEC adherence factor plasmid (pEAF) that encodes bundle-forming pili and regulatory genes, while a subset of EPEC strains, known as atypical EPEC (aEPEC) lack the EAF plasmid.

EPEC strains are a well-recognized cause of infantile diarrhea in developing countries, whereas EHEC strains, mainly serotype O157:H7, are responsible for a wide spectrum of clinical diseases including diarrhea, hemorrhagic colitis and hemolytic uremic syndrome (HUS), the leading cause of renal failure in children in Argentina and several other countries (12). Healthy cattle are postulated as

a major source of human EHEC O157:H7 infections, although other healthy and diarrheic animals can carry EHEC strains belonging to different serotypes (15). Although the O157:H7 serotype is the prototype of highly pathogenic EHEC, non-Shiga toxin-producing *E. coli* O157 strains have been isolated from children with diarrhea (3), but their role as commensal or pathogenic bacteria is not completely clear. Most of these *E. coli* serogroup O157 isolates were categorized as EPEC or aEPEC. In this study, we analyze Shiga toxin-negative *E. coli* O157 strains previously isolated as part of an epidemiological survey of Shiga toxin-producing *E. coli* O157 in dogs in Argentina (1). All the isolates were obtained from the same district in Buenos Aires suburbs along one year.

A total of eleven *E. coli* O157 strains belonging to different H-types were investigated for additional virulence markers and clonal relationships. The *eae* gene was subtyped by PCR-RFLP analysis as described by Ramachandran *et al.* (11). The *bfpA* gene that encodes the bundle-forming pilus was amplified by PCR as described (6). Classification of the *E. coli* strains into the four major phylogenetic groups (A, B1, B2 and D) was carried out by using the multiplex PCR protocol proposed by Clermont *et al.* (4). The macrorestriction fragment separation by PFGE of the *E. coli* O157:H16 strains was performed by using the 24-h PulseNet standardized PFGE protocol for *E. coli* O157:H7, with minor modifications (5). Cluster analysis of the electrophoretic profiles obtained by PFGE was carried out with

the BioNumerics version 3.5 software package (Applied Maths, Belgium) using the Dice coefficient and UPGMA to generate dendrograms with 1.5% tolerance values. The strains were characterized by the pattern of adherence to HEp-2 cells in the presence of D-mannose according to the method described by Scaletsky *et al.* (13). Fermentation of sorbitol and production of enterohemolysin were carried out as previously described (2).

The characteristics of *E. coli* O157 strains identified in this study are shown in Table 1. The *eae* gene was present in all except one of the O157:H16 strains and in the O157:H45 strain. Further characterization of the *eae* gene revealed that the O157:H16 and O157:H45 strains examined were intimin subtype ϵ and α_1 , respectively. Only the *E. coli* O157:H45 strain harbored the *bfpA* gene. In contrast to most O157:H7 strains, all the isolates fermented sorbitol and were enterohemolysin negative. Concerning the adhesion of bacterial isolates to HEp-2 cells, all *E. coli* O157:H16 *eae* positive strains showed a localized adherence-like (LAL) pattern, while the O157:H45 strain adhered to HEp-2 cells in a localized manner (LA), a phenotype associated with the expression of bundle-forming pili. The O157:H29 strain was non-adherent. A total of eight different *Xba*I-PFGE patterns with more than 74% similarity were identified among the nine *E. coli* O157:H16 strains. Two O157:H16 strains isolated from epidemiologically non-related animals showed the same *Xba*I-PFGE patterns (Figure 1).

Table 1. Characterization of non-Shiga toxin-producing *E. coli* O157 strains

Serotype	n	<i>stx</i> ₁	<i>stx</i> ₂	<i>eae</i>	<i>bfpA</i>	Phylogenetic group	Adherence pattern	Pathotype
O157:H16	8	-	-	ϵ	-	A	LAL ⁽¹⁾	aEPEC
	1	-	-	-	-	A	NA ⁽²⁾	-
O157:H29	1	-	-	-	-	B1	NA	-
O157:H45	1	-	-	α_1	+	B2	LA ⁽³⁾	EPEC

⁽¹⁾No adherence; ⁽²⁾Localized adherence-like; ⁽³⁾Localized adherence

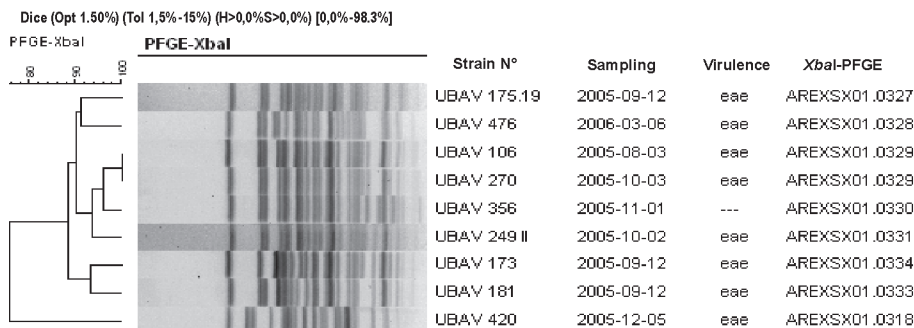


Figure 1. PFGE of *E. coli* O157:H16 strains isolated from dogs.

Most typical EPEC serotypes have been isolated only from humans, whereas several animal hosts, including dogs, harbor atypical EPEC that belong to a wide diversity of serotypes (8). A significant association between aEPEC and diarrhea in children has been observed (10). In this study, we found that the *E. coli* O157:H16 isolates fell within the aEPEC pathotype, because all of them lacked the *bfp* gene. Furthermore, when these strains were analysed by PFGE, all but two strains displayed genotypic patterns with more than 90% similarity and two strains were indistinguishable. This result shows the highly genetic relationship between the strains of this serotype, which seems to be widespread among dogs in the neighbourhoods of Buenos Aires city. Moreover, *E. coli* O157:H16 strains with the same virulence profile have been found to be associated with bloody and non-bloody diarrhea in children, and have also been detected in sausage and hamburger samples in Argentina (M. Rivas, INEI-ANLIS, personal communication). In comparison, the *E. coli* O157:H45 strain seems to be a typical EPEC. EPEC O157:H45, produced a massive outbreak of diarrhea in humans in Japan in 1998 (7).

Phylogenetic analyses have shown that the population structure of *E. coli* is clonal and the *E. coli* strains fall into four main phylogenetic groups (A, B1, B2 and D) (4). In accordance with previous studies (14), we observed an association between the different O157 serotypes and phylogenetic ancestry. Each *E. coli* serotype belonged to a distinct phylogenetic group, A, B1 and B2, indicating the emergence of O157 strains in the three different lineages. The O157:H7 lineage is proposed to have emerged from an O55:H7-like EPEC ancestor by shifting the O-antigen from O55 to O157 (14). Both serotypes possess the *eae* gene encoding γ subclass intimin. In contrast, *E. coli* O157:H45 possesses α_1 subclass intimin, which is present in the typical EPEC O55:H6.

In conclusion, our results confirm that dogs may harbour pathogenic *E. coli* O157 which do not correspond to Shiga toxin-producing strains and whose potential human health hazard should not be underestimated.

Acknowledgements: This work was supported by PIP grant N° 6556 from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). D.A. Vilte is a PhD student supported by the Instituto Nacional de Tecnología Agropecuaria. M. Larzábal is a PhD student and A. Cataldi is a CONICET fellow, respectively. We are indebted to Ana María Elizondo and Laura González for their invaluable technical assistance. The HEp-2 cells were gently provided by Dr. Osvaldo Zábal from the Instituto Nacional de Tecnología Agropecuaria.

REFERENCES

- Bentancor A, Rumi MV, Gentilini MV, Sardoy C, Irino K, Agostini A, *et al.* Shiga toxin-producing and attaching and effacing *Escherichia coli* in cats and dogs in a high hemolytic uremic syndrome incidence region in Argentina. *FEMS Microbiol Lett* 2007; 267: 251-6.
- Beutin L, Montenegro MA, Orskof I, Orskov F, Prada J, Zimmermann S, *et al.* Close association of verotoxin (Shiga-like toxin) production with enterohemolysin production in strains of *Escherichia coli*. *J Clin Microbiol* 1989; 27: 2559-64.
- Blank TE, Lacher DW, Scaletsky ICA, Zhong H, Whittam TS, Donnenberg MS. Enteropathogenic *Escherichia coli* O157 strains from Brazil. *Emerg Infect Dis* 2003; 9: 113-5.
- Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Appl Environ Microbiol* 2000; 66: 4555-8.
- Foodborne and Diarrheal Diseases Branch, Centers for Disease Control and Prevention Standardized Molecular Subtyping of Foodborne Bacterial Pathogens by Pulsed-field Gel Electrophoresis: A Training Manual. Centers for Disease Control and Prevention, 1998 Atlanta, GA USA.
- Gunzburg ST, Tornieporth NG, Riley LW. Identification of enteropathogenic *Escherichia coli* by PCR-based detection of the bundle-forming pilus gene. *J Clin Microbiol* 1995; 35: 1375-7.
- Makino S, Asakura H, Shirahata T, Ikeda T, Takeshi K, Arai K, *et al.* Molecular epidemiological study of a mass outbreak caused by enteropathogenic *Escherichia coli* O157:H45. *Microbiol Immunol* 1999; 43: 381-4.
- Nakazato G, Gyles C, Ziebell K, Keller R, Trabulsi LR, Gomes TA, *et al.* Attaching and effacing *Escherichia coli* isolated from dogs in Brazil: characteristics and serotypic relationship to human enteropathogenic *E. coli* (EPEC). *Vet Microbiol* 2004; 101: 269-77.
- Nataro JP, Kaper JB. Diarrheagenic *Escherichia coli*. *Clin Microbiol Rev* 1998; 11: 42-201.
- Nguyen RN, Taylor LS, Tauschek M, Robins-Browne RM. Atypical enteropathogenic *Escherichia coli* infection and prolonged diarrhea in children. *Emerg Infect Dis* 2006; 12: 597-603.
- Ramachandran V, Brett K, Hornitzky MA, Downton M, Bettelheim KA, Walker MJ, *et al.* Distribution of intimin subtypes among *Escherichia coli* isolates from ruminant and human sources. *J Clin Microbiol* 2003; 41: 5022-32.
- Repetto HA. Long-term course and mechanisms of progression of renal disease in haemolytic uremic syndrome. *Kidney Int* 2005; S10: 2-6.
- Scaletsky ICA, Silva MLM, Trabulsi LR. Distinctive patterns of adherence of enteropathogenic *Escherichia coli* to HeLa cells. *Infect Immun* 1984; 45: 534-6.
- Tarr PI, Schoening LM, Yea YL, Ward TR, Jelacic S, Whittam TS. Acquisition of the *rfb-gnd* cluster in evolution of *Escherichia coli* O55 and O157. *J Bacteriol* 2000; 182: 6183-91.
- Yoon JW, Hovde CJ. All blood, no stool: enterohemorrhagic *Escherichia coli* O157:H7 infection. *J Vet Sci* 2008; 9: 219-31.